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Functional and behavioral metrics for evaluating laser retinal damage

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ABSTRACT

The use of lasers by both the military and civilian community is rapidly expanding. Thus, the potential for and severity of laser eye injury and retinal damage is increasing¹. Sensitive and accurate methods to evaluate and follow laser retinal damage are needed. The multifocal electroretinogram (mfERG) has the potential to meet these criteria. In this study, the mfERG was used to evaluate changes to retinal function following laser exposure. Landolt C contrast acuity was also measured in the six behaviorally trained Rhesus monkeys. The monkeys then received Nd:YAG laser lesions (1064 nm, 9 ns pulse width) in each eye. One eye received a single foveal lesion of approximately 0.13 mJ total intraocular exposure (TIE) and the other received six parafoveal lesions which varied in TIE from 0.13 to 4 mJ. mfERGs and behavioral data were collected both pre- and post-exposure. mfERGs were recorded using stimuli that contained 103, 241, and 509 hexagons. Landolt C contrast acuity was measured with five sizes of Landolt C (0.33 to 11.15 cycles/degree) of varying contrast. mfERG response densities were sensitive to the functional retinal changes caused by the laser insult. In general, larger lesions showed greater mfERG abnormalities than smaller laser lesions. Deficits in contrast acuity were found to be more severe in the eyes with foveal injuries. Although the mfERG and contrast acuity assess different areas of the visual system, both are sensitive to laser-induced retinal damage and may be complementary tests for laser eye injury triage.

Keywords: multifocal electroretinogram, mfERG, laser retinal injury, nonhuman primate, contrast sensitivity, visual acuity

1. INTRODUCTION

The use of lasers on the modern battlefield is expanding. Military uses of lasers include target range determination, target designation, illumination and high-energy weapons among others. Most of these lasers are of infrared (IR) or visible wavelengths. In addition, laser illumination of commercial airline pilots has been reported in recent years²⁻⁴. Thus far, all reports have indicated that commercial airline illuminations have been with visible lasers. Whether from military or civilian sources, lasers of these wavelengths, IR and visible, can cause serious injury to the eye, specifically the retina. The severity of a retinal injury depends on a number of factors affiliated with the power of the laser and the distance from the laser when illuminated, such as the area of the retina illuminated, the energy absorbed by the retina, and the wavelength and pulse duration of the laser. A small injury to the peripheral retina might go unnoticed to the exposed person and would not likely have any serious complications that would compromise vision in the future. Conversely, a large lesion to the fovea can have immediate and dire consequences for vision⁵⁻⁷.

There are two primary ways to determine if a person has had a retinal injury after exposure to a laser. The first is based on the patient's symptoms that might include seeing floaters, reduced acuity, and Amsler grid distortion. The second way to diagnose a laser-caused retinal injury is via ophthalmoscopy. Examination of an injured retina with an

ophthalmoscope might reveal retinal lesions, retinal scars, retinal hemorrhage, and possibly vitreous hemorrhage. The multifocal electroretinogram (mfERG) is a relatively new technique that objectively tests focal function of the central retina. Using a pseudorandom flickering on a monitor, hundreds of responses from the central retina can be recorded^{8,9}. The mfERG has been shown to be sensitive to retinal changes that occur in many diseases including age-related macular degeneration, diabetic retinopathy, retinitis pigmentosa, and retinal detachment¹⁰. mfERG response characteristics have been shown to vary depending on the part of the retina that is affected by a disease or injury¹¹. The mfERG therefore has the potential to be a sensitive and objective method to assess patients who have sustained laser eye injury.

In the current study, we tested contrast acuity and recorded mfERGs in nonhuman primates before and after exposing each eye with an Nd:YAG (1064 nm) laser. Results are presented and the potential for using the mfERG to evaluate laser retinal injuries is discussed.

2. METHODS

2.1 Animal Preparation: Six Rhesus monkeys (8.0 – 11.5 kg) housed under standard laboratory conditions (12 hours light/12 hours dark) were used in this study. All animals involved in this study were procured, maintained, and used in accordance with Army Regulation 40-33, the Federal Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council. The Air Force Research Laboratory (AFRL) at Brooks City-Base, Texas, has been fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International, (AAALAC) since 1967. Using a standard protocol reported elsewhere^{12,13}, all animals received local (bupivacaine/lidocaine peribulbar injections) and general anesthesia (propofol via syringe pump) to stabilize eye movements for the laser exposures and for mfERG recordings.

2.2 Laser Exposures: All animals received one laser exposure of approximately 0.11 to 0.32 mJ total intraocular exposure (TIE) to the fovea of the non-dominant eye (as determined by prior contrast acuity testing). The other eye received six parafoveal lesions which varied in TIE from 0.13 to 4 mJ depending on location. (see Table 1). Exposure numbers (Exp #) 1 and 2 were applied inferior to the fovea, 3 and 4 temporal to the fovea, and 5 and 6 superior to the fovea. Exp # 7 was applied to the fovea of the other eye. One animal (D26Z) received an additional four test lesions inferior to the fovea prior to applying the six parafoveal lesions. A vitreous hemorrhage was caused by one of the test lesions but the hemorrhage remained in the inferior vitreous during the follow-up (Day 0) mfERG testing. In addition, a vitreous hemorrhage was caused in one animal (D10Z) by the application of the foveal lesion.

Table 1.

Exp #	Average TIE (mJ)	Standard Deviation (mJ)	Retinal Location
1	3.58	0.53	Parafovea (inf)
2	3.79	0.38	Parafovea (inf)
3	0.34	0.03	Parafovea (temp)
4	0.33	0.03	Parafovea (temp)
5	0.19	0.06	Parafovea (sup)
6	0.19	0.06	Parafovea (sup)
7	0.21	0.10	Fovea

2.3 Multifocal Electroretinogram Recordings: Baseline and post-laser exposure mfERGs were recorded on the animals on Day 0 (about one hour after laser exposure), Day 5, Week 3, and Week 7. Stimulus arrays of 103, 241, and 509 unscaled hexagons were used for recordings at each session. Only the 241 hexagon results are presented here (Fig. 1). Custom manufactured Burian-Allen electrodes (Hansen Ophthalmic Development Lab, Coralville, IA) were placed on the anesthetized (proparacaine drops) corneas of the anesthetized animal subjects for the recording process. mfERGs were collected from the subjects using the Visual-Evoked Response Imaging System (VERIS™, Electro-Diagnostic Imaging, Redwood City, CA). An infrared camera allowed for continuous visualization of the posterior segment of the eye during mfERG recordings. The VERIS system stimulus pattern was projected onto the fundus and the optics were

located approximately 4 cm from the cornea. Using unscaled stimuli, 103, 241, and 509 focal ERG response recordings were recorded from each eye during each session. The luminance of a hexagon when white was approximately 300 cd/m² and when black was as dark as possible yielding a contrast of nearly 100%. Using the VERIS system software for artifact removal, filtering, and the combining of left and right eyes, normal files were created for each animal using that animal's baseline 241 hexagon recordings. With these normal files, mfERG response density plots were evaluated both pre- and post-laser exposure and any signs of deviation from normal noted.

2.3 Behavioral Testing: Numerous pre- and post-laser exposure contrast acuity measurements were collected on each animal. After initial acclimation to the test environment, the animals were trained on a series of tasks that were of increasing complexity over a series of several months. The animal was elevated to the next most difficult task once the animal had achieved an accuracy of approximately 90% for the less difficult task. The final pre-exposure acuity/contrast sensitivity measurements were recorded after approximately two months of training on this most difficult task level. For each acuity measurement session, an animal was placed in a chair 80 centimeters (cm) from a display monitor. A soft, neoprene mask was placed over the animal's eyes and used to occlude the opposite eye for monocular recordings. Five gap sizes of a Landolt "C" (0.33, 0.98, 3.98, 6.31, and 11.15 cycles/degree) were tested and a psychophysical staircase (gradual lightening of the "C" with a reduction in size) was used to control the contrast. Animals held down a lever until they saw the "C" at which point they released the lever. Correct responses received within 5 seconds were rewarded. A Probit¹⁴ function (frequency of seeing curve or "S-shaped" curve) was fit to the percent correct data for each "C" size, for each animal, and for each session. The 50% correct point on the Probit function was used as the threshold contrast for each "C" size.

3. RESULTS

Figure 2 shows the TIE applied to animal D26Z's right (foveal) and left (6 parafoveal and 4 test lesions) retinas. The drawings illustrate the approximate location of the lesions and the photographs show the post-exposure appearance of the lesions. The right fovea received a single laser exposure to the fovea of very low energy and the lesion is barely visible on the retinal photograph. One of the test lesions applied to the inferior retina of the left eye (OS1) caused an immediate vitreous hemorrhage. The parafoveal lesions in the left eye can be seen in the second OS photograph (OS2).

The baseline mfERG 3D response density plot from D26Z's left eye is shown in Figure 3. Response density is essentially response amplitude. Large changes in waveform timing can affect response density. The top, lower left and lower right plots are referred to as the "Difference," the "Subject," and the "Reference" plots, respectively. The view is a "Field" view which has the superior aspect of the retina on the bottom of the image and the inferior aspect of the retina on the top half of the image. The subject plot (Fig. 3 lower left) shows the animal's baseline response. An area with reduced responses showing the optic disc (blind spot) is visible in the left, slightly inferior portion of the subject plot. The reference plot (bottom right) is composed of baseline recordings from both eyes. The reference plot is somewhat bumpy reflecting the fact that the subject's baseline recording from the right eye was somewhat noisy. This is also reflected in the difference plot (upper) that appears to have some areas of depression, especially in the fovea. This is considered the normal condition for these baseline and reference recordings.

Figure 4 shows D26Z's five day post-exposure mfERG recording. The subject plot shows greater central depression compared with the baseline and the small foveal peak seen in the baseline plot is gone. The area where the test lesions were administered and vitreous hemorrhage occurred (inferior retina/superior field) shows a large area with reduced responses (Fig. 4, "Difference" plot).

Figure 5 shows the baseline and follow-up mfERG recording "Difference" plots for D26Z. Although D26 Z received only a small, barely visible lesion to the right fovea, all follow-up plots show reduced response densities centrally compared with the combined normals files. The left eye responses are reduced in the inferior aspect of the retina (superior aspect of plot on a field view) corresponding to the area where the test lesions were applied, although there appears to be recovery in the later recordings (D. and E.).

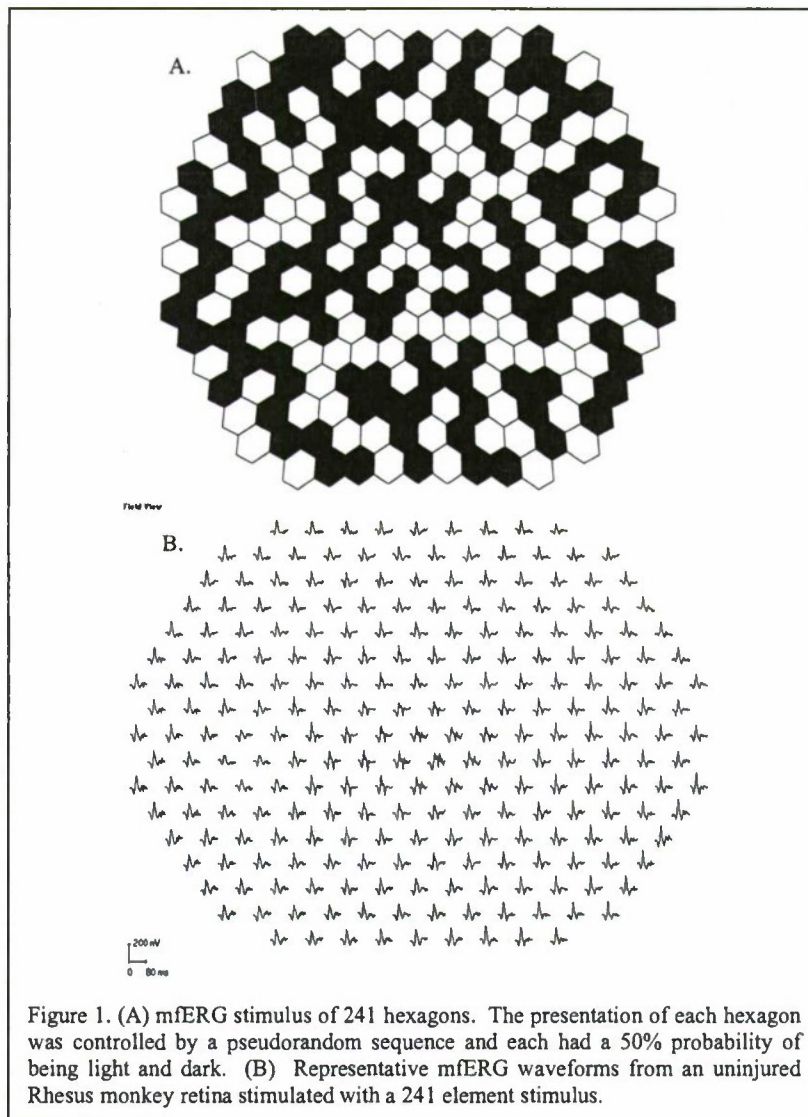


Figure 1. (A) mfERG stimulus of 241 hexagons. The presentation of each hexagon was controlled by a pseudorandom sequence and each had a 50% probability of being light and dark. (B) Representative mfERG waveforms from an uninjured Rhesus monkey retina stimulated with a 241 element stimulus.

Treatment date: 5/5/2005; D26Z;
1064 nm laser, 8 - 9 ns pulse width

Exp #	Power (calc)	
1, 2	3.78, 3.9	mJ
3, 4	0.32, 0.32	mJ
5, 6	0.16, 0.16	mJ
7	0.11	mJ

Test Shots	Power		
T1	3.41	mJ	no immed lesion
T2	4.27	mJ	no immed lesion
T3	6.28	mJ	Hemorrhage
T4	0.32	mJ	

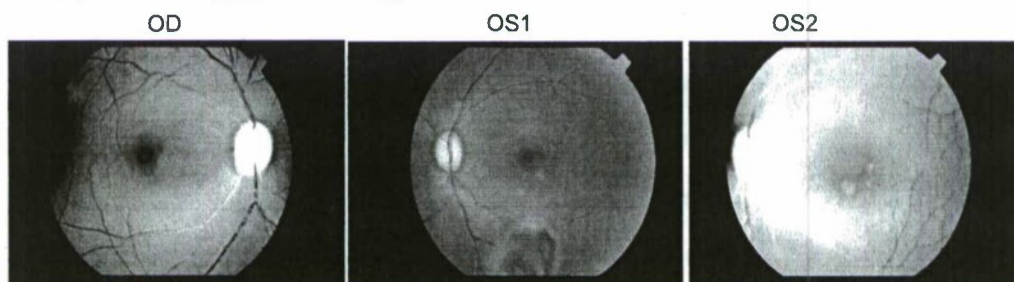
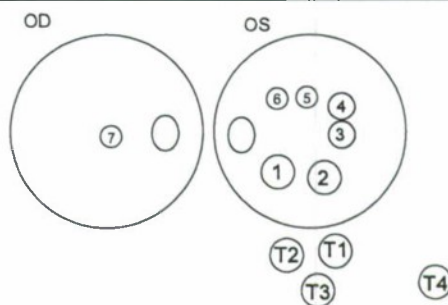


Figure 2. Laser TIEs to the right eye (#7) and left eye (#1 - #6). Four test lesions were applied to the inferior left retina. The drawings show the location of the lesions and the photographs show post-exposure lesions.

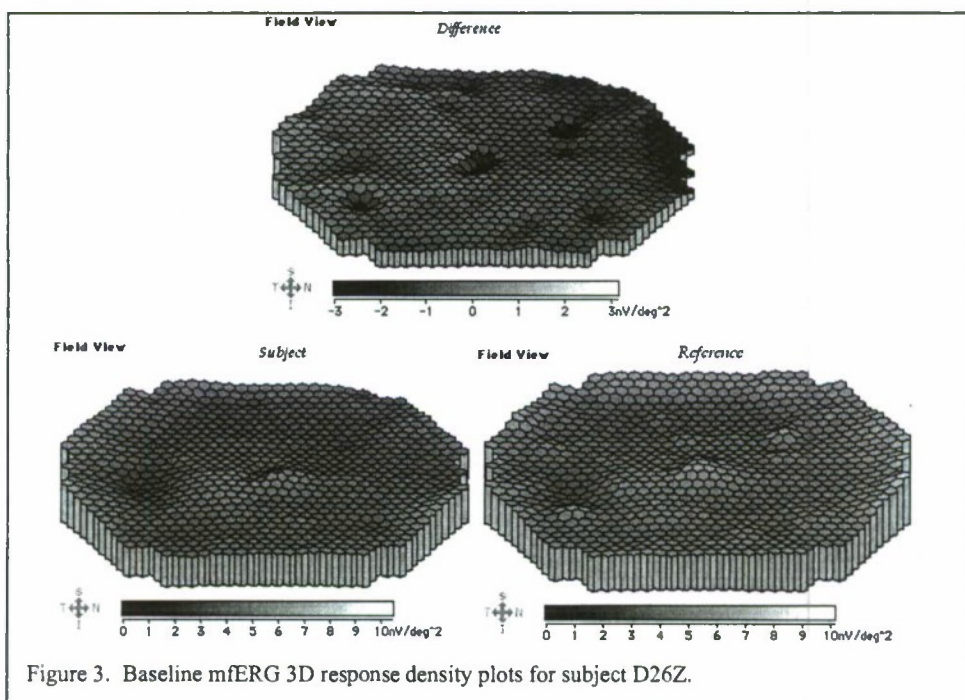


Figure 3. Baseline mfERG 3D response density plots for subject D26Z.

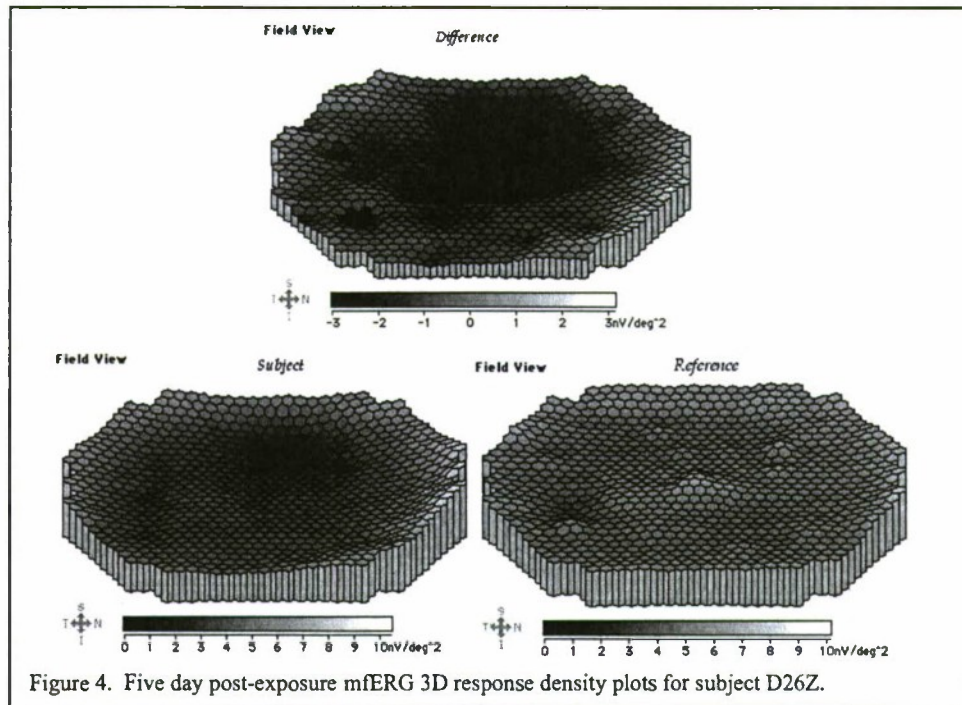


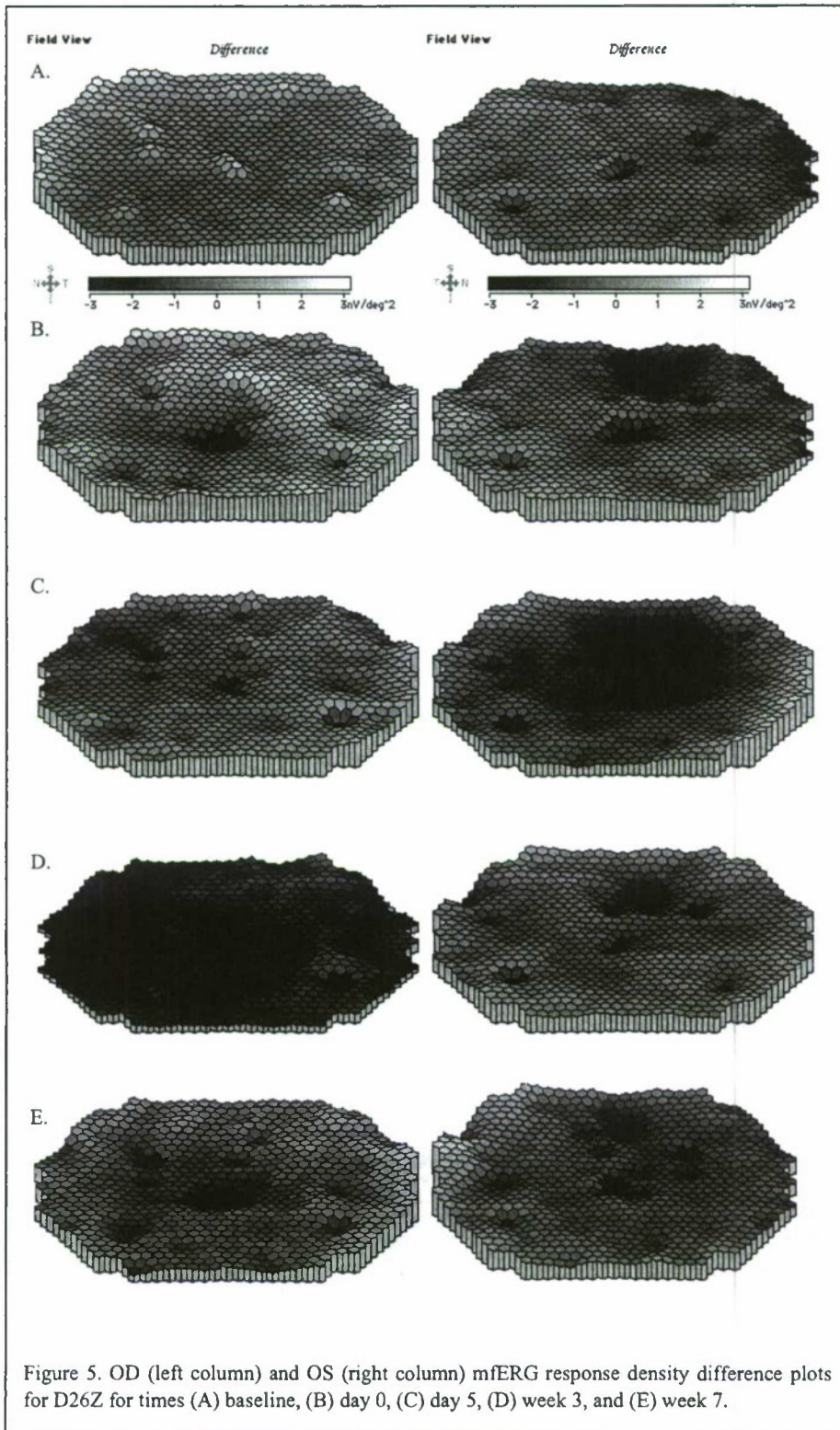
Figure 4. Five day post-exposure mfERG 3D response density plots for subject D26Z.

The average behavioral results for D26Z are shown in Figure 6. For each eye, the post-exposure results are reduced compared with the pre-exposure results (Paired T-test; $p < 0.05$). Generally, post-exposure responses are reduced more at low- and mid-spatial frequencies and are relatively normal at the highest spatial frequency. Although on average D26Z's post-exposure responses were reduced, individual responses were not always reduced. Overall for the right eye, 10 of 13 individual recordings were reduced at all spatial frequencies. For the left eye, 10 of 12 post-exposure measurements were reduced at all spatial frequencies. Thus, on an individual basis, some of D26Z's behavioral results do not show a deficit at some or all spatial frequencies.

Figure 7 shows the five day post-exposure laser lesions from D10Z. A large vitreous hemorrhage was caused by the foveal laser exposure to the left eye and the lesion can be clearly seen in the right eye. The 6 parafoveal lesions in the left eye are clearly visible in the retina. The mfERG response density difference plots for D10Z at baseline, 5 day post-exposure, and 3 week post-exposure are shown in Figure 8. The large foveal lesion in the right eye is clearly evident in the post-exposure plot. However, the large parafoveal lesions that are evident in the photograph of the left eye are not easily seen in the mfERG responses.

Despite having a large foveal lesion and reduced mfERG responses, D10Z's contrast acuity was significantly better after the laser lesions were applied (Fig. 9). Post-exposure behavioral results were generally better than pre-exposure results.

Overall, five of six subjects that received foveal laser exposures showed reduced central responses on their mfERG results. Only two of six subjects with parafoveal lesions showed definite mfERG reductions. For the behavioral results, only two of six animals who received foveal lesions had statistically reduced average contrast acuities. For animals with parafoveal lesions, two of six also displayed reduced contrast acuities on average. Individual post-exposure contrast acuity results varied widely from better to worse than pre-exposure results.



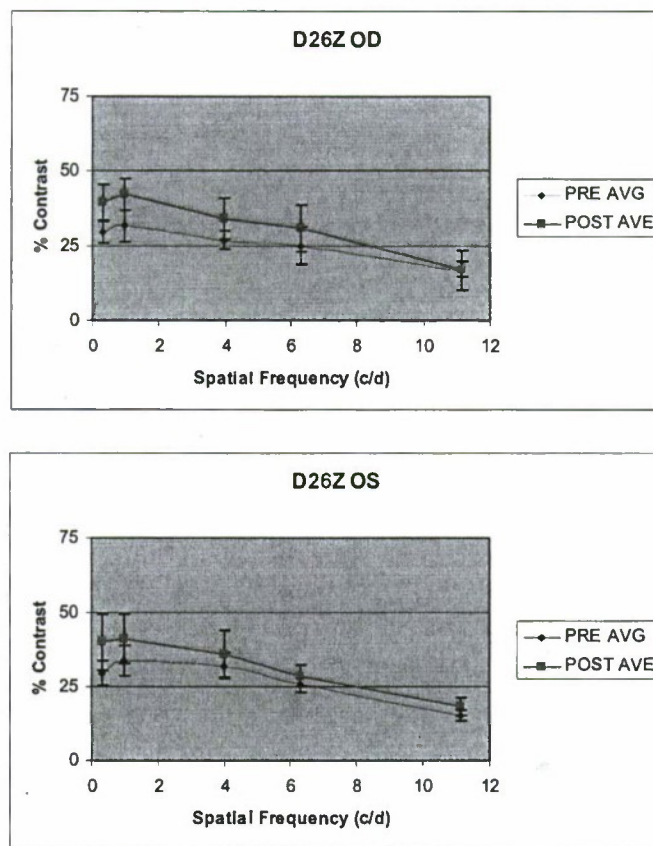


Figure 6. Average pre- and post-exposure contrast acuity results for the right eye (upper) and left eye (lower) for D26Z. Shown are results of each of the 5 "C" gap sizes. Contrast results are for the 50% correct values from the Probit functions. Error bars show ± 1 standard deviation.

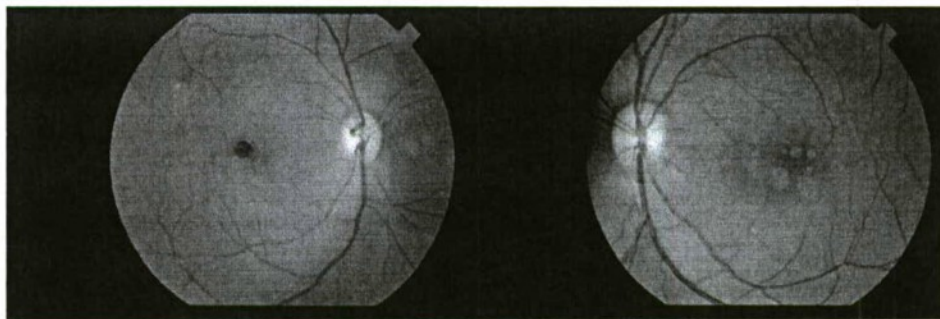


Figure 7. Day 5 post-exposure laser lesions for D10Z. The OD (left) shows a large, central retinal hemorrhage that was previously a vitreous hemorrhage. The OS (right) shows six parafoveal lesions.

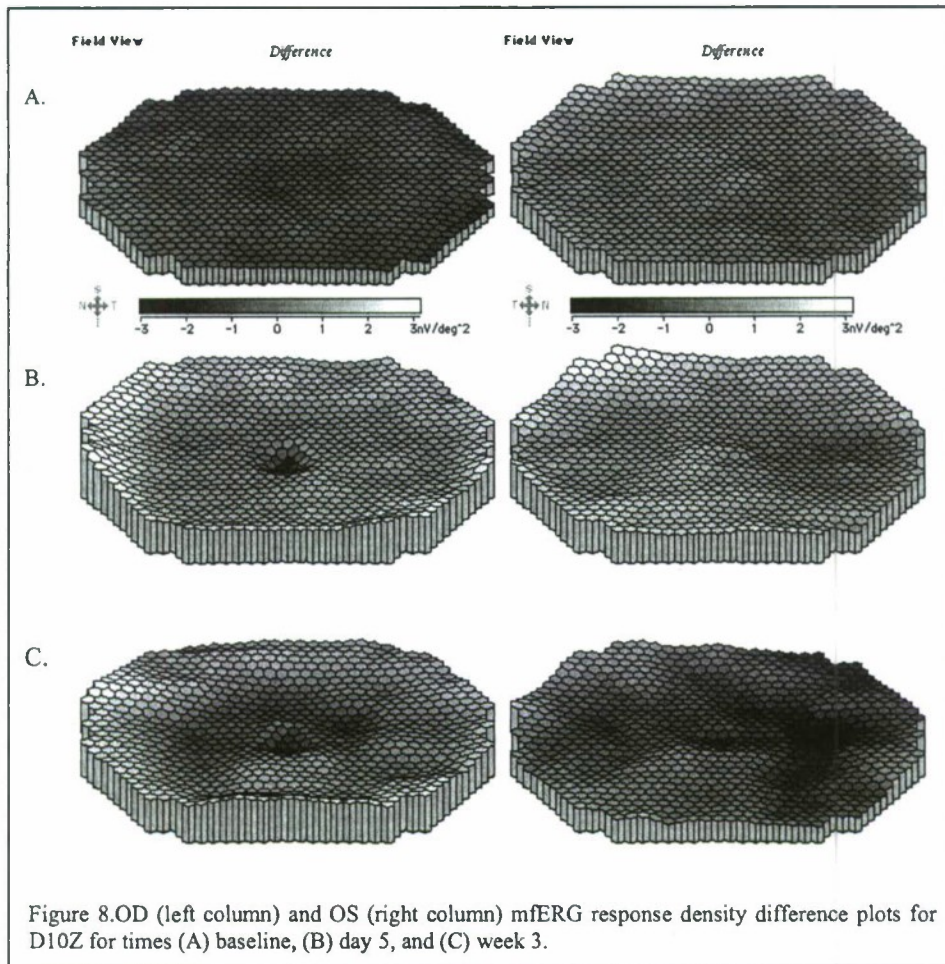


Figure 8. OD (left column) and OS (right column) mfERG response density difference plots for D10Z for times (A) baseline, (B) day 5, and (C) week 3.

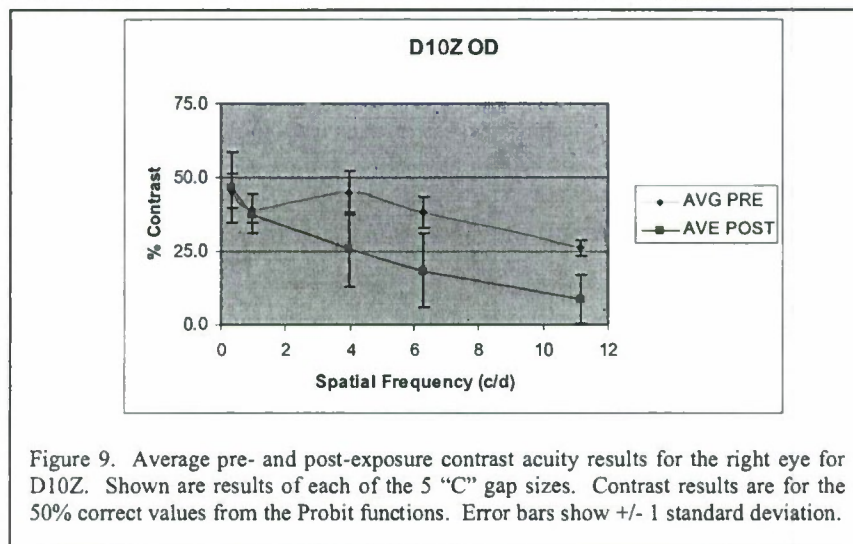


Figure 9. Average pre- and post-exposure contrast acuity results for the right eye for D10Z. Shown are results of each of the 5 "C" gap sizes. Contrast results are for the 50% correct values from the Probit functions. Error bars show ± 1 standard deviation.

4. CONCLUSIONS

The monkey has proven to be a useful model to evaluate retinal function and changes with the mfERG^{15, 16}. The mfERG may also prove valuable in detecting and following laser retinal injuries. The mfERG has been found to be sensitive to large lesions from an argon laser¹⁷ and to secondary laser damage⁷. The current study found readily visible mfERG response reductions in four of six eyes with foveal exposures. Less definitive results were found in eyes with parafoveal lesions. Additional data exploration is required to quantify the overall sensitivity of the mfERG to detect laser retinal injuries in the nonhuman primate model.

Fewer than half of the laser treated eyes showed reduced contrast acuity after laser exposure. Individually, many post-exposure measurements produced better acuity than pre-exposure tests. The reasons for this are not clear but may include inadequacies in the testing and evaluation software, the improvement of the animal's performance to accurately complete a task over time and the ability of the visual system to fill in the retinal gaps where the damage occurred. Additionally, acuity testing in the non-human primate is a difficult task and may sometimes be too variable to produce reliable results.

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